

IN THE SPECIFICATION

Please replace the paragraph beginning at page 2, line 8 with the following amended paragraph:

The present invention features a novel ~~calcium-bind~~ calcium-binding protein hereinafter designated HCBP and characterized as having similarity to a calcium-binding protein, reticulocalbin.

Please replace the paragraph beginning at page 4, line 16 with the following amended paragraph:

“Consensus”, as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, or which has been extended using an XL-PCR kit (~~Perkin Elmer~~ PERKIN ELMER, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been assembled from the overlapping sequences of more than one ~~Incyte~~ INCYTE clone using the GELVIEW fragment assembly system (GCG, Madison, WI), or which has been both extended and assembled.

Please replace the paragraph beginning at page 10, line 1 with the following amended paragraph:

Nucleic acids encoding the human HCBP of the present invention were first identified in ~~Incyte~~ Clone INCYTE clone 2509570 from a sigmoid mesentery tumor tissue cDNA library (CONUTUT01) through a computer-generated search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: ~~Incyte Clones~~ INCYTE clones 1852086 (LUNGFET03), 2111063 (BRAITUT03), 2214592

(SINTFET03), 1878487 (LEUKNOT03), 2309791 (NGANN0T01), and 2509570 (CONUTUT01).

Please replace the paragraph beginning at page 12, line 19 with the following amended paragraph:

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE polymerase (US Biochemical Corp, Cleveland, OH), Taq polymerase (~~Perkin Elmer~~) (PERKIN ELMER), thermostable T7 polymerase (~~Amersham~~ AMERSHAM, Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System amplification system marketed by ~~Gibco BRL~~ GIBCO BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the ~~Hamilton~~ MICROLAB 2200 system (~~Hamilton~~ HAMILTON, Reno, NV), Peltier Thermal Cycler (~~PTC200, MJ~~ PTC200 thermal cycler (MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (~~Perkin Elmer~~) (PERKIN ELMER).

Please replace the paragraph beginning at page 13, line 20 with the following amended paragraph:

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries to walk in genomic DNA (~~Clontech~~ CLONTECH, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences

which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Please replace the paragraph beginning at page 13, line 30 with the following amended paragraph:

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. GENOTYPER and SEQUENCE NAVIGATOR, Perkin Elmer PERKIN ELMER) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Please replace the paragraph beginning at page 15, line 3 with the following amended paragraph:

In another embodiment, sequences encoding HCBP may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223, Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of HCBP, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis

may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer) peptide synthesizer (PERKIN ELMER).

Please replace the paragraph beginning at page 16, line 5 with the following amended paragraph:

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene STRATAGENE, LaJolla, CA) or PSORT1 plasmid (Gibco BRL) (GIBCO BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding HCBP, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

Please replace the paragraph beginning at page 16, line 20 with the following amended paragraph:

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for HCBP. For example, when large quantities of HCBP are needed for the induction of

antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as BLUESCRIPT (Stratagene) phagemid (STRATAGENE), in which the sequence encoding HCBP may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega PROMEGA, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

Please replace the paragraph beginning at page 20, line 16 with the following amended paragraph:

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HCBP include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HCBP, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, Kalamazoo MI; Promega PROMEGA, Madison WI; and U.S. Biochemical Corp., Cleveland OH). Suitable reporter molecules or labels, which may be

used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Please replace the paragraph beginning at page 20, line 29 with the following amended paragraph:

Host cells transformed with nucleotide sequences encoding HCBP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HCBP may be designed to contain signal sequences which direct secretion of HCBP through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding HCBP to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immuno
IMMUNEX Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen INVITROGEN, San Diego, CA) between the purification domain and HCBP may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing HCBP and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281) while the enterokinase cleavage site provides a means for purifying HCBP from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

Please replace the paragraph beginning at page 21, line 19 with the following amended paragraph:

In addition to recombinant production, fragments of HCBP may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using ~~Applied Biosystems~~ APPLIED BIOSYSTEMS 431A Peptide Synthesizer (~~Perkin Elmer~~) peptide synthesizer (PERKIN ELMER). Various fragments of HCBP may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

Please replace the paragraph beginning at page 35, line 28 with the following amended paragraph:

The frozen tissue was homogenized and lysed in Trizol TRIZOL reagent (1 gm tissue/10 ml Trizol TRIZOL; Cat. #10296-028; ~~Gibco/BRL~~ GIBCO BRL, Gaithersburg, MD), a monoplastic solution of phenol and guanidine isothiocyanate, using a Brinkmann Polytron POLYTRON PT-3000 homogenizer (Brinkmann Instruments, Westbury, NY). After a brief incubation on ice, chloroform was added (1:5 v/v) and the lysate was centrifuged. The upper chloroform layer was removed to a fresh tube and the RNA extracted with isopropanol, resuspended in DEPC-treated water, and DNase treated for 25 min at 37°C. The RNA was re-extracted twice with acid phenol-chloroform pH 4.7 and precipitated using 0.3M sodium acetate and 2.5 volumes ethanol. The mRNA was then isolated using the QIAGEN OLIGOTEX kit (QIAGEN, Inc., Chatsworth, CA) and used to construct the cDNA library.

Please replace the paragraph beginning at page 36, line 6 with the following amended paragraph:

The mRNA was handled according to the recommended protocols in the SUPERSCRIPT Plasmid System for cDNA Synthesis and Plasmid Cloning plasmid system for cDNA synthesis and plasmid cloning (Cat. #18248-013, Gibco/BRL GIBCO BRL). The cDNAs were fractionated on a Sepharose SEPHAROSE CL4B column (Cat. #275105-01; Pharmacia), and those cDNAs exceeding 400 bp were ligated into pINCY1. The plasmid pINCY1 was subsequently transformed into DH5a competent cells (Cat. #18258-012; Gibco/BRL GIBCO BRL).

Please replace the paragraph beginning at page 36, line 14 with the following amended paragraph:

Plasmid DNA was released from the cells and purified using the REAL Prep 96 Plasmid Kit PREP 96 plasmid kit (Catalog #26173, QIAGEN, Inc.). This kit enabled the simultaneous purification of 96 samples in a 96-well block using multi-channel reagent dispensers. The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, Gibco/BRL GIBCO BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4°C.

Please replace the paragraph beginning at page 36, line 23 with the following amended paragraph:

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton MICROLAB 2200 system (Hamilton HAMILTON, Reno, NV) in combination with Peltier Thermal Cylers (PTC200 from MJ PTC200 thermal cyclers (MJ Research, Watertown, MA) and Applied Biosystems APPLIED BIOSYSTEMS 377 DNA Sequencing Systems sequencing systems; and the reading frame was determined.

Please replace the paragraph beginning at page 36, line 29 with the following amended paragraph:

After the reading frame was determined, the nucleotide sequences of the Sequence Listing or amino acid sequences deduced from them were used as query sequences against databases such as GenBank GENBANK, SwissProt, BLOCKS, and Pima II. These databases which contain previously identified and annotated sequences, were searched for regions of homology (similarity) using BLAST, which stands for Basic Local Alignment Search Tool (Altschul (1993) supra, Altschul (1990) supra).

Please replace the paragraph beginning at page 37, line 21 with the following amended paragraph:

Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank GENBANK or the LIFESEQ database (Incyte INCYTE Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

Please replace the paragraph beginning at page 38, line 10 with the following amended paragraph:

Nucleic acid sequence of ~~Incyte~~ INCYTE clone 2509570 or SEQ ID NO:2 is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

Please replace the paragraph beginning at page 38, line 25 with the following amended paragraph:

By following the instructions for the XL-PCR kit (~~Perkin Elmer~~) (PERKIN ELMER) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (~~PTC200, M.J.~~ PTC200 thermal cycler (MJ Research, Watertown, MA) and the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min

Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

Please replace the paragraph beginning at page 40, line 10 with the following amended paragraph:

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 μ Ci of [γ -³²P] adenosine triphosphate (~~Amersham~~) (AMERSHAM) and T4 polynucleotide kinase (DuPont NEN, Boston, MA). The labeled oligonucleotides are substantially purified with SEPHADEX G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10^7 counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II; DuPont NEN).

Please replace the paragraph beginning at page 40, line 21 with the following amended paragraph:

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (~~Nytran~~ NYTRAN Plus, ~~Schleicher & Schuell~~ SCHLEICHER & SCHUELL, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR film (~~Kodak~~ KODAK, Rochester, NY) is exposed to the blots, or the blots are exposed to a ~~Phosphoimager~~ PHOSPHORIMAGER cassette (~~Molecular Dynamics~~ MOLECULAR DYNAMICS, Sunnyvale, CA), hybridization patterns are compared visually.

Please replace the paragraph beginning at page 42, line 13 with the following amended paragraph:

Typically, the oligopeptides are 15 residues in length, synthesized using an ~~Applied Biosystems Peptide Synthesizer Model~~ APPLIED BIOSYSTEMS model 431A ~~peptide synthesizer~~ using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, ~~Sigma~~ SIGMA, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.